human reproduction

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Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies

Jane L. Halliday[1,2,7], Obioha C. Ukoumunne[1,2], H.W. Gordon Baker[3,4], Sue Breheny[5], Alice M. Jaques[1], Claire Garrett[4], David Healy[5,6], and David Amor[1,2,3]

[1]Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Rd, Parkville, VIC 3052, Australia [2]Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia [3]Melbourne IVF, East Melbourne, VIC, Australia [4]Department of Obstetrics and Gynaecology, University of Melbourne, Royal Women's Hospital, Parkville, VIC, Australia [5]Monash IVF, Richmond, VIC, Australia [6]Department of Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia

[7]Correspondence address. Tel: +61-3-8341-6260; Fax: +61-3-8341-6212; E-mail: janehalliday.h@mcri.edu.au

BACKGROUND: The reasons for increased birth defect prevalence following *in-vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are largely unknown. Classification of birth defects by pathology rather than organ system, and examination of the role of embryo freezing and thawing may provide clues to the mechanisms involved. This study aimed to investigate these two factors.

METHOD: Data on 6946 IVF or ICSI singleton pregnancies were linked to perinatal outcomes obtained from population-based data sets on births and birth defects occurring between 1991 and 2004 in Victoria, Australia. These were compared with 20 838 outcomes for singleton births in the same population, conceived without IVF or ICSI. Birth defects were classified according to pathogenesis.

RESULTS: Overall, birth defects were increased after IVF or ICSI [adjusted odds ratio (OR) 1.36; 95% CI: 1.19–1.55] relative to controls. There was no strong evidence of risk differences between IVF and ICSI or between fresh and thawed embryo transfer. However, a specific group, blastogenesis birth defects, were markedly increased [adjusted OR 2.80, 95% CI: 1.63–4.81], with the increase relative to the controls being significant for fresh embryo transfer (adjusted OR 3.65; 95% CI: 2.02–6.59) but not for thawed embryo transfer (adjusted OR 1.60; 95% CI: 0.69–3.69).

CONCLUSION: Our findings suggest that there is a specific risk of blastogenesis birth defects arising very early in pregnancy after IVF or ICSI and that this risk may be lower with use of frozen-thawed embryo transfer.

Key words: assisted reproduction / birth defects / blastogenesis / embryo transfer / cryopreservation

Introduction

Assisted reproductive technologies (ART) have been found to be associated with an increase in birth defects, many studies of which are now summarized in systematic reviews (Rimm *et al.*, 2004; Hansen *et al.*, 2005). With over 200 000 babies born worldwide each year by ART (de Mouzon *et al.*, 2009; Dobson, 2009) and the number of cycles in Europe (Andersen *et al.*, 2008) and the USA (Wright *et al.*, 2008) steadily growing, a significant increase in birth defects amongst these subpopulations is likely to pose a substantial burden not only on the families involved, but also on the health care systems. Importantly, controversy remains regarding whether

these studies of birth defects have been biased by ascertainment due to the more stringent follow-up of ART-conceived babies, a lack of proper controls, or an insufficient sample size (Schieve *et al.*, 2005; Cohen, 2007). Therefore, further research has been necessary to provide a clearer understanding of the possible mechanisms associated with the noted increase in birth defects and to address the ascertainment issue as much as possible.

Our study was designed to examine the prevalence of birth defects after assisted reproduction in a geographically defined population, with a very high quality Birth Defects Register. We chose to use a classification system based on a framework which groups together birth defects that are likely to have similar mechanistic causes (Wellesley

et al., 2005), one that was different from the routinely used International Classification of Diseases (ICD) system which groups birth defects by body systems.

We were specifically interested in a category of birth defects that have previously been classified as 'defects of blastogenesis' (Opitz, 1993). 'Blastogenesis' in this context refers to the period of human development from fertilization up until the end of gastrulation, corresponding to first 4 weeks of embryo development. As a group, blastogenesis defects occur prior to organogenesis and tend to affect the formation of the midline and mesoderm, involve two or more developmental fields, be severe, and be without sex differences in occurrence. They include defects of fusion, lateralization, segmentation, morphogenic movement and asymmetry. Blastogenesis defects are usually of unknown aetiology; however, these defects are more often sporadic and with a low empiric recurrence risk (Opitz, 1993). We noted that the category of blastogenesis defects includes several specific defects that have been observed to be increased in ART pregnancies in previous studies: neural tube defects, abdominal wall defects, esophageal atresia and anal atresia (Kallen *et al.*, 2005; Reefhuis *et al.*, 2008). Monozygotic twinning, another type of blastogenesis defect, has also been observed to occur more frequently in ART pregnancies (Aston *et al.*, 2008). On the basis of these findings from other studies, we hypothesized that exposures related to ovarian stimulation, oocyte collection and embryo culture were most likely to impact upon the early development of the embryo, rather than exerting an effect later in pregnancy. Our first aim was to specifically examine the prevalence of blastogenesis defects.

We further hypothesized that the cryopreservation status of the embryo at the time of transfer would influence the risk of birth defects following ART. This was because some studies, recently systematically reviewed (Wennerholm *et al.*, 2009), have observed better perinatal outcomes for thawed embryo transfer compared with fresh embryo transfer (Sutcliffe *et al.*, 1995; Olson *et al.*, 2005; Belva *et al.*, 2008). As our study was sufficiently powered, our second aim was to undertake a subgroup analysis of birth defects based on cryopreservation status.

Materials and Methods

This retrospective record linkage cohort study used three data sets: (i) The ART database was obtained from Melbourne IVF and Monash IVF, the two independent service providers that between them undertook more than 98% of procedures in the study period (1991–2004) in the State of Victoria, Australia. The data from these services were merged, then extensive data cleaning and standardization of variable formatting were required; (ii) The Perinatal Data Collection Unit (PDCU) is a mandatory collection of all births in Victoria, Australia, of 20 weeks gestation and later (Riley *et al.*, 2005). There were approximately 64 000 births each year in the study period. Midwives used a standard form to report data relating to pregnancy, birth episode and the newborn; (iii) The Birth Defects Register (BDR) is a subset of PDCU data supplemented by notifications from multiple sources and contains data on birth defects from all gestations, including terminations for birth defects (Riley and Halliday, 2006). In 1991, approximately 6% of all birth defects that were notified to the BDR were terminations before 20 weeks gestation and by 2004 this had risen to 12%. Validation studies using medical records as the gold standard have demonstrated complete ascertainment of structural defects apparent at birth (including ones associated with chromosome

disorders) and 89% ascertainment of terminations with little difference between public (87%) and private hospitals (91%) (Riley *et al.*, 2001, 2004).

Study groups and record linkage

Overall, there were 9355 singleton ART pregnancies from 1991 to 2004 in Victoria. The perinatal outcomes were established through computerized, probabilistic record linkage of ART data to the PDCU birth and BDR data. Inclusion criteria for the study were: *in-vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures, non-donor oocytes, single heartbeat and gestational sac at the 6 weeks ultrasound followed by singleton birth after 19 weeks gestation or termination of pregnancy for a birth defect at any gestation, and fresh embryo transfer after ovarian stimulation with follicle stimulation hormone or frozen embryo transfer in a natural menstrual cycle. There were 6946 ART pregnancies that met the above inclusion criteria.

Non-ART controls were singleton births or terminations for a birth defect, selected from the same state-wide population-based PDCU/BDR data, which originally included the ART births but these were excluded from the dataset prior to control selection. Controls were frequency matched on maternal age and baby year of birth, on a three controls to one ART ratio, giving a total of 20 838 controls. Including both the ART and non-ART records, there were 1350 (5%) women who had two or more children and the sibship clustering was accounted for in the statistical analysis.

Ethics approval

This study was approved by Human Research Ethics Committees that governed the various data collections, and allowed for record linkage without informed consent. The committees were from Victorian Department of Human Services, Mercy Health and Aged Care, Royal Women's Hospital, Freemasons Hospital, Epworth Hospital, Monash University and Monash Surgical Private Hospital.

Birth defect classification systems

We devised a novel classification system based on a previously published hierarchical system (Wellesley *et al.*, 2005). We first grouped birth defects with known chromosomal, genetic, teratogenic and syndromic causes and then assigned all others to one of three groups: blastogenesis defects, other multiple malformations or isolated malformations. Importantly, all classification was done by a medical expert blinded to the ART conception status. Each fetus/baby with a birth defect was assigned to only one of the following categories.

Disorders with known aetiology: (i) chromosome disorders and microdeletion syndromes. Because some chromosome disorders would not be apparent at birth, but would be detected by prenatal testing, chromosome disorders were subdivided according to whether or not there would be a phenotype at birth (e.g. mosaics and sex chromosome abnormalities were excluded); (ii) disorders caused by exposure to a teratogen; (iii) monogenic disorders; (iv) imprinting disorders such as Beckwith–Wiedemann syndrome, a rare condition, but known to be more common in ART conceptions (Amor and Halliday, 2008).

Disorders with unknown aetiology: (i) Disorders of blastogenesis, (Opitz, 1993) defined as the presence of one or more of the following: abdominal wall defects, vertebral segmentation defects, tracheoesophageal fistula, diaphragmatic defects, neural tube defects, anal atresia, renal agenesis, caudal regression sequence, laterality defects, sirenomelia, sacrococcygeal teratoma, holoprosencephaly, acro-renal field defect and amelia; (ii) Other multiple malformations were those with two or more birth defects in two or more body systems; (iii) All isolated birth defects not previously classified above, including more than one birth defect in one

body system, and more than one defect occurring as part of a sequence. The most frequent isolated birth defects were cardiac defects, hypospadias, undescended testes, talipes, developmental dysplasia of the hip, obstructive uropathies (e.g. hydronephrosis, renal cystic dysplasia), cleft lip and/or palate, digital abnormalities, brain abnormalities. A heterogeneous category of 'all other' abnormalities comprised isolated birth defects occurring in smaller numbers, less than 20 cases.

Statistical analysis

Quantitative data were summarized using means and standard deviations and categorical data were summarized using numbers and percentages. Logistic regression was used to compare the odds of a birth defect between the ART and non-ART controls. In addition, the odds of birth defects were compared between IVF and ICSI and between fresh and thawed embryo transfer. Odds ratios (ORs) are presented for the subgroups of ART relative to the non-ART controls. Odds were also compared between ART and non-ART controls for specific types of birth defect, but only for those where there were at least 70 cases overall to have sufficient numbers for the logistic regression to provide stable estimates. Unadjusted analyses and analyses adjusted for the well-recognized potential confounders of birth defects, maternal age, sex of baby and parity were implemented. A measure of time, baby year of birth, was also included in the adjusted analyses as overall ascertainment of birth defects has improved over the study period.

The ORs were estimated using the method of marginal models fitted using Generalized Estimating Equations (GEE) with information sandwich ('robust') estimates of standard error to allow for the correlation between the results related to births from the same mother (Hanley et al., 2003). An 'exchangeable' correlation structure was specified for the GEE analyses.

Results

Table I summarizes the characteristics of the study population. The original matching ensured no differences between the non-ART and combined ART groups for maternal age and year of baby birth. The differences, however, in year of baby birth and parity between the ART study subgroups underscore the importance of inclusion of these variables as potential confounders in adjusted analyses. There was a small amount of missing data on parity and sex as this was not always reported in the termination records.

Table II shows the results of the unadjusted and adjusted analyses of birth defects. Birth defects were reported in 1003 conceptions without ART (4.8%; 95% CI: 4.5–5.1%) and in 443 conceived by ART (6.4% 95% CI: 5.8–6.9%). Compared with the non-ART group, the adjusted OR of a birth defect after any ART procedure was 1.36 (95% CI: 1.19–1.55; $P < 0.001$). There was, however, no strong evidence of a difference between the ORs for IVF and ICSI (adjusted OR for ICSI to IVF = 1.19; 95% CI: 0.96–1.48), or between the ORs for fresh and thawed embryo transfer (adjusted OR for fresh to thawed embryo transfer = 1.15; 95% CI: 0.93–1.43).

Types of birth defects

Table III shows the specific types of birth defects detected. They are presented according to the hierarchical classification system described in Materials and Methods section. Adjusted ORs for specific birth defects with more than 70 cases reported are shown in Table IV for ART relative to the non-ART controls. Birth defects for which there were less than 70 cases were: Beckwith–Wiedemann syndrome, two or more disorders in two or more systems, digital abnormalities, cleft lip and/or palate and brain abnormalities. In addition, analyses were not performed for the 'all other' isolated category because of its heterogeneity. Some examples from the 'all other' isolated category with more than five cases were Hirschsprung disease, other intestinal malformations, congenital hypothyroidism, naevus, craniosynostosis, cardiomyopathy and pyloric stenosis. No

Table I Characteristics of women and their singleton births in the study groups defined by assisted reproduction status

	Non-ART control N = 20 838	ART All ART (N = 6946)	P*	IVF (N = 3312)	ICSI (N = 3634)	P**	Fresh ET (N = 4323)	Thawed ET (N = 2623)	P#
Age of mother in years, mean (SD)	34.0 (4.1)	33.9 (4.1)	1	34.1 (4.1)	33.9 (4.2)	0.03	33.9 (4.2)	34.0 (4.0)	0.27
Year of baby birth, mean (SD)	1999.5 (3.6)	1999.5 (3.6)	0.99	1998.2 (4.0)	2000.7 (2.8)	<0.001	1999.6 (3.5)	1999.3 (3.8)	<0.001
Parity (%)									
0	30.1	64.9		62.6	67.0		69.9	56.6	
1–2	56.0	32.2		34.1	30.6		27.3	40.3	
3+	13.2	2.1		2.4	1.8		1.9	2.4	
Unknown	0.7	0.7	<0.001	0.9	0.5	<0.001	0.8	0.6	<0.001
Sex (%)									
Male	51.0	51.6		53.5	49.8		51.9	51.0	
Female	48.8	48.2		46.2	50.1		47.9	48.8	
Unknown	0.15	0.18	0.77	0.24	0.14	0.04	0.21	0.15	0.57

ET, embryo transfer.
*P difference between non-ART and all ART.
**P difference between IVF and ICSI.
#P difference between fresh ET and thawed ET.

Table II Odds Ratios for all birth defects for ART compared with non-ART, IVF compared with ICSI and thawed ET compared with fresh ET

Group	N	% Birth defects (n)	Unadjusted OR (95% CI)	Adjusted# OR (95% CI)
Non-ART controls	20 838	4.8 (1003)	Reference	Reference
ART	6946	6.4 (443)	1.35 (1.20–1.51)##	1.36 (1.19–1.55)##
IVF	3312	6.3 (208)	1.33 (1.14–1.55)	1.31 (1.10–1.56)
ICSI	3634	6.5 (235)	1.37 (1.18–1.58)	1.40 (1.19–1.65)
Fresh ET	4323	6.7 (292)	1.43 (1.25–1.64)	1.43 (1.23–1.66)
Frozen ET	2623	5.8 (151)	1.21 (1.01–1.44)	1.25 (1.04–1.52)
IVF			Reference	Reference
ICSI			1.05 (0.86–1.28)	1.19 (0.96–1.48)
Thawed ET			Reference	Reference
Fresh ET			1.18 (0.96–1.45)	1.15 (0.93–1.43)

ET, embryo transfer.
#All analyses adjusted for maternal age, year of baby birth, parity and sex.
##P-value for comparison between ART combined and non-ART controls is <0.001.

Table III Number and percentage of specific birth defects for non-ART controls and ART

n (%)	Non-ART (N = 20 838)	ART (N = 6946)
Birth defects with known aetiology		
All chromosome disorders	203 (0.97)	69 (0.99)
Chromosome disorders evident at birth	167 (0.80)	48 (0.69)
Monogenic disorders	59 (0.28)	20 (0.29)
Beckwith–Wiedemann syndrome	2 (0.01)	3 (0.04)
Birth defects with unknown aetiology		
Blastogenesis defects	54 (0.25)	43 (0.62)
Two or more abnormalities in two or more systems	42 (0.20)	21 (0.30)
Isolated abnormalities		
Hypospadias	57 (0.27)	26 (0.37)
Undescended testes	85 (0.40)	31 (0.45)
Obstructive uropathies	88 (0.42)	26 (0.37)
Congenital heart disease	128 (0.61)	51 (0.73)
Development dysplasia of hip (DDH)	49 (0.23)	33 (0.47)
Talipes	52 (0.25)	27 (0.39)
Digital abnormalities	28 (0.13)	16 (0.23)
Cleft lip and/or palate	24 (0.11)	13 (0.19)
Brain abnormalities	21 (0.10)	8 (0.11)
All others	105 (0.50)	56 (0.81)

patterns were obvious on examination of the numbers of these less common disorders between the ART and non-ART groups.

No strong evidence of increases for the ART group was seen for any birth defects with known chromosomal or other genetic aetiology.

A breakdown into chromosome abnormalities seen at birth, excluding mosaics and sex chromosome abnormalities, did not demonstrate any strong evidence of increased odds, nor was there an increase seen for monogenic disorders. There were no disorders identified as being due to teratogen exposure.

In the first unknown aetiology classification category in Table III, disorders of blastogenesis, there were 43 (0.62%; 95% CI: 0.45–0.83%) birth defects in the ART group, compared with 54 (0.25%; 95% CI: 0.19–0.34%) in the non-ART group. Many of the blastogenesis defects were in pregnancies that were terminated, with proportionately more in the non-ART group than in the ART group [37% (20/54) and 26% (11/43), respectively]. The OR comparing ART to non-ART was 2.80 (95% CI: 1.63–4.81) after adjusting for maternal age, baby year of birth, parity and sex (Table IV). To ensure that records of terminations were not excluded from the analysis because of missing data on parity, and because blastogenesis defects have no sex differences in terms of prevalence, a sensitivity analysis was implemented, restricting adjustment to just maternal age and year of birth. The resulting OR was 2.43 (95% CI: 1.63–3.63), a slightly smaller effect.

The adjusted OR for the direct comparison between IVF and ICSI showed no difference (OR 0.92; 95% CI: 0.38–2.23) in risk of defects of blastogenesis. However, the fresh and thawed embryo transfer comparison groups demonstrated a difference with an OR of 2.41 (95%CI: 1.03–5.65). Table IV shows the adjusted ORs for IVF and ICSI, fresh embryo transfer and thawed embryo transfer, compared with the non-ART group. In the first three of these subgroups a significant 2- to 4-fold increase was evident for blastogenesis defects, but such an increase was not seen for thawed embryo transfer (OR 1.60 (95% CI: 0.69–3.69). There were 28 blastogenesis defects associated with IVF, 15 with ICSI, 33 with fresh embryo transfer and only 10 with thawed embryo transfer.

The blastogenesis defects associated with ART were malformations seen in isolation (N = 27) or as part of multiple malformations (N = 16), four of which had more than one blastogenesis defect. They

Table IV Adjusted Odds Ratios (95% CIs) comparing all ART, IVF and ICSI, fresh and thawed embryo transfers, to non-ART controls (reference category) for specific birth defects

	All ART	IVF	ICSI	Fresh ET	Thawed ET
All chromosome disorders	1.09 (0.71–1.68)	0.89 (0.49–1.61)	1.29 (0.77–2.15)	1.11 (0.66–1.87)	1.06 (0.59–1.93)
Chromosome disorders evident at birth	0.86 (0.49–1.51)	0.80 (0.38–1.67)	0.92 (0.46–1.85)	0.73 (0.35–1.51)	1.06 (0.52–2.16)
Monogenic disorders	0.89 (0.51–1.56)	1.22 (0.64–2.33)	0.57 (0.24–1.36)	0.78 (0.39–1.54)	1.07 (0.50–2.30)
Blastogenesis defects	2.80 (1.63–4.81)	3.24 (1.79–5.86)	2.33 (1.12–4.87)	3.65 (2.02–6.59)	1.60 (0.69–3.69)
Isolated birth defects:					
Hypospadias	1.56 (0.93–2.62)	1.93 (1.03–3.60)	1.23 (0.63–2.41)	1.35 (0.72–2.50)	1.90 (0.98–3.68)
Undescended testes	1.09 (0.72–1.65)	1.10 (0.63–1.90)	1.09 (0.62–1.90)	1.37 (0.86–2.18)	0.66 (0.30–1.43)
Obstructive uropathies	0.98 (0.62–1.54)	0.40 (0.16–1.00)	1.50 (0.90–2.48)	0.91 (0.52–1.59)	1.09 (0.58–2.06)
Congenital Heart Disease	1.25 (0.88–1.79)	1.13 (0.69–1.84)	1.35 (0.89–2.07)	1.16 (0.75–1.78)	1.40 (0.87–2.26)
Developmental dysplasia of hip	1.41 (0.87–2.27)	1.21 (0.63–2.33)	1.57 (0.91–2.70)	1.52 (0.89–2.60)	1.21 (0.60–2.44)
Talipes	1.30 (0.81–2.09)	1.44 (0.78–2.62)	1.17 (0.64–2.17)	1.52 (0.89–2.58)	0.93 (0.42–2.06)

All analyses adjusted for maternal age, year of baby birth, parity and sex.

included 12 neural tube defects, eight oesophageal atresia or VATER association, eight renal agenesis, five congenital diaphragmatic hernia, five anal atresia, three exomphalos, two situs inversus, two acro-renal field defect, one hemifacial microsomia and one caudal regression.

For all other categories of isolated birth defects, there was no strong evidence of any differences between the ART and non-ART groups, but there was a suggestion of an increase in hypospadias with IVF.

Discussion

Our study found an overall increase in birth defects after ART (6.4%) compared with non-ART pregnancies (4.8%), with an adjusted OR of 1·36. There were 6946 IVF and ICSI records, including 2623 thawed embryo transfers, making the study large enough to examine IVF and ICSI separately, as well as consider the possible effect of the cryopreservation status of the embryo. We found no strong evidence of a difference in overall risk of birth defects between IVF and ICSI, or between fresh embryo transfer and thawed embryo transfer, but close examination of specific types of birth defects revealed new important information. By using a classification system other than the routinely used ICD system, which groups birth defects into body systems, we found a specific association between ART and the almost invariably severe blastogenesis birth defects arising in the first 4 weeks of pregnancy. They were present in 1 in 160 ART pregnancies compared with 1 in 400 controls. They are rare defects but they are not as rare as the imprinting disorders (Amor and Halliday, 2008), which have raised concerns for service providers and authorities and attracted a lot of media attention and consumer interest.

Strengths and limitations

Our findings were obtained having excluded pregnancies with more than one fetal heart in the ART population to eliminate adverse perinatal outcomes associated with the vanishing twin syndrome (La Sala et al., 2006; Pinborg et al., 2007). Such exclusion was not possible in the control population, which may therefore have had cases in which there were vanishing twins, and led to an underestimation of the true OR. The overall results (OR 1.36), however, are in the higher range of ORs reported in previous meta-analyses that demonstrate a pooled OR of 1.29 (Rimm et al., 2004; Hansen et al., 2005). This may be because of the high quality of the data collected by the Victorian Birth Defects Register with multiple sources of ascertainment, including a large number of pregnancy terminations for birth defects before 20 weeks gestation.

Bias in ascertainment of the blastogenesis birth defects in ART pregnancies is unlikely because they are typically severe or lethal, and apparent before or at birth. Importantly, there were proportionately more terminations identified amongst the non-ART group, which challenges the frequent criticism of studies of ART and birth defects on the basis that the ART group is more closely followed.

Limitations of this study were the inability to examine aetiology of infertility or compare data with a group of sub-fertile women to examine the effect of infertility per se on birth defects.

Length of involuntary childlessness, as a proxy for sub-fertility, was examined in a large Swedish study (Kallen et al., 2005) and seemed to account for much of the risk of birth defects along with the confounders we included, such as maternal age and parity. It is, however, difficult to compare with this study as it did not include pregnancy terminations. It remains possible that there may be some specific associations with these potential risk factors that we have not identified (Farhi and Fisch, 2007). Nevertheless, the overriding finding in this study showing the increased risk for blastogenesis defects stands alone.

Interpretation of findings

Blastogenesis disorders are typically sporadic and without known genetic cause, consistent with a possible environmental aetiology, such as might be associated with the ART process. The increase in blastogenesis defects appears greater for fresh embryo transfer than for thawed embryo transfer with the risk for fresh embryo transfer relative to controls being more than 3-fold. This risk may be explained by the cryopreservation process acting as a 'selection gate' for more

viable embryos (Michelmann and Nayudu, 2006). Alternatively, or in addition, the excessive ovarian hormonal exposures related to oocyte collection, which occurs immediately prior to fresh embryo transfer, but not before thawed embryo transfer, could have a variety of adverse effects on the very early pregnancy (Shih *et al.*, 2008). An abnormal hormonal milieu would not have been present immediately before implantation of the thawed embryo transfer in this study, as we included only those transferred in a normal menstrual cycle. In the case of fresh embryo transfer, it is possible that endometrial receptivity is compromised in the presence of the high hormone levels that persist beyond the time of oocyte collection. Some evidence for this is the lower level of endometrial protein, pregnancy associated plasma protein A (PAPP-A), in pregnancies where there has been fresh embryo transfer, but not with thawed embryo transfer (Amor *et al.*, 2009). This is a relevant finding in this context because PAPP-A plays a key role in angiogenesis and placentation in the early weeks of pregnancy.

In regards to other birth defects, this study found no increase in genetic abnormalities, in agreement with a study of de novo gene mutations in mice fetuses produced by ART (Caperton *et al.*, 2007), but contrary to studies suggesting more chromosome abnormalities with ICSI (Bonduelle *et al.*, 2002; Katalinic *et al.*, 2004). The only slight evidence of increases were for musculoskeletal abnormalities and hypospadias, as has been suggested in other studies (Hansen *et al.*, 2002; Olson *et al.*, 2005; Zhu *et al.*, 2006), but no increases were as strong as that seen for the blastogenesis disorders. Aside from the use of a different classification system, our different results may be explained by exclusion of twins and pregnancies with more than one fetal heart on early ultrasound.

Conclusion

By using a classification system that describes birth defects on the basis of the developmental phase in which they arise, we identified defects of blastogenesis as the only ones for which there was strong evidence of an increase in ART pregnancies. This suggests a mechanism initiated about the time of implantation, affecting early embryo development. Future research into the association between ART and birth defects should use an aetiological framework to describe birth defects and examine the possible influence of the cryopreservation process itself and the exposures related to oocyte collection and embryo transfer on birth defect prevalence.

Authors' Roles

J.L.H. formulated the research questions, performed statistical analyses, results interpretation and wrote the article. O.C.U. devised the data analysis plan and provided expert statistical advice. A.M.J. prepared all data, performed the record linkage and edited the article. S.B. and C.G. provided the data from the clinics, assisted with interpretation of the results and edited the article. D.L.H. and H.W.G.B. oversaw the project, assisted with the interpretation of results and edited the article. D.J.A. developed the classification and undertook coding (blinded to ART status), played a major role in interpretation of results and in writing the article.

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